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THE INFLUENCE OF CERTAIN ADDED SOLIDS UPON THE COMPOSITION AND EFFICIENCY OF KNOP'S NUTRIENT SOLUTION

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It was pointed out many years ago by Nägeli (1) that such insoluble substances as filter paper and paraffin shavings have the power of rendering water from a copper still suitable for the culture of algae. This observation led True and Oglevee (2) to make a study of the effect of these and other similar substances on the growth of plants in various solutions, the toxicity of which had previously been determined. They found that solutions of copper sulfate, silver nitrate, and other substances, in concentrations near their toxic limits, were rendered markedly less toxic or even stimulating to lupine radicles when accompanied by such substances as shredded filter paper, freshly prepared potato starch and fine sea sand in amounts equal to about one third that of the culture solution. These authors comment on the adsorbing power of soils and its relation to plant growth.

Breazeale (3) studied the question from another angle. To soil extracts which supported but a poor growth of seedlings were added such substances as washed carbon black, calcium carbonate, ferric hydrate, aluminum hydrate, and quartz flour. Ferric hydrate was especially effective in causing an increased development of the roots of wheat seedlings. It was also found that these solids have a remarkable power of suppressing the toxic properties of distilled water. Parker (4) has measured the power of soils to adsorb salts from solutions and found not only that the concentrations of salts may be changed, but also that there is selective adsorption of certain ions from the solution.

Consideration of these and related observations led the writers to investigate the effects upon the physiological balance of a nutrient solution, of various added solids supposed to offer relatively large presentations of surface. It was deemed possible that, if changes in the solution occurred under these conditions, the evidence obtained might render possible a distinction between adsorptive effects and ordinary chemical reactions, as causal agents. Rather unsuspected relations have been disclosed by the results obtained, which seem to justify their publication as matters of general interest.

METHODS OF INVESTIGATION

Young seedlings of Chevalier barley and Little Gem peas were grown, according to the method previously described by Tottingham (5), in pint jars containg Knop's solution[1] of about 0.3 percent total concentration in which had been incorporated a definite amount of a watery suspension of the solid to be studied. Six plants were grown in each jar, and each modification of the solution was employed in duplicate. The substances added to the usual nutrient solution were, respectively: silicic acid, ferric hydrate, and carbon black.

The silicic acid was prepared by adding slowly, with constant stirring, the required amount of dilute HCl to a cold, dilute solution of sodium silicate. Preliminary tests had shown this to give the most satisfactory material. The gelatinous precipitate was washed with water, by decantation, until the clear washing fluid gave no test for chlorides. Ferric hydrate was prepared by dissolving ferric oxide in hot, dilute nitric acid. The ferric nitrate solution thus formed was diluted, precipitated cold with dilute ammonia water until neutral to litmus, and the precipitated hydrate was washed by decantation until free from nitrate. "G-Elf" brand of carbon black was washed with dilute hydrochloric acid and then repeatedly washed with hot water until the wash water no longer gave visible evidence of impurities resembling oil or tar. In each case the solid was allowed to settle over night in water; then the clear, upper layer of water was siphoned away and the actual concentration of the thoroughly agitated suspension of each substance was determined by evaporating aliquot portions. In view of the uncertainty regarding the composition of the precipitated ferric hydrate, as noted by Mendeleeff (6), the iron was determined as Fe_2O_3 by ignition, and from this the equivalent amount of $Fe(OH)_3$ was calculated. The evaporated residue from the silicic acid suspension was ignited and weighed as SiO_2. The nutrient solutions for each jar were made up as needed from stock solutions of the separate salts of M/2 concentration; the watery suspension was incorporated in making the solution up to volume.

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[1] The composition of the solution is shown by the number of cc. of M/2 solution of each salt used per liter of solution: $Ca(NO_3)_2$, 20.9; $MgSO_4$, 7.1; K_2HPO_4, 2.5; KH_2PO_4, 2.5; KNO_3, 8.5. Equal parts of KH_2PO_4 and K_2HPO_4 were used for the purpose of approximating neutrality more closely than obtains where but one of these salts is used. Four drops of colloidal ferric phosphate per jar were added with each fresh portion of solution.

Two series of cultures were run for each material. In one series, there was incorporated in each 400 cc. portion of nutrient solution the equivalent of 2.5 grams of dry substance of the solid to be added. In the other, the equivalent of 0.5 grams of dry substance was used. The relative fineness of division of the solids is indicated by the following volumes of each suspension required to supply 2.5 grams of dry substance: H_2SiO_3, 93.5 cc.; $Fe(OH)_3$, 85.6 cc.; carbon black, 18.3 cc.

Thrifty seedlings about five days old, selected to be as uniform in size and appearance as possible, were supported in jars by the method used by Tottingham (5). About two thirds of the pea cotyledons were removed to hasten the effect of the nutrient solution. The plants were grown in a greenhouse at Madison, Wis., from July 20 to August 10, 1914, the cultures being shaded from the intense sunlight of the period by a cheesecloth curtain. Daily aeration of the solutions was accomplished by means of an atomizer bulb, and the solutions were renewed every three days. At the end of the growth period of twenty-one days, the plants were removed and the residual nutrient solutions, after being restored to their original volumes, were tested for hydrogen-ion concentration. Later, fresh nutrient solutions were prepared to determine their initial acidity and to test for any possible adsorption after contact with the solids.

In analyzing the culture solutions, the suspensions were allowed to settle, the clear liquids were filtered off, and aliquot parts were taken for the various determinations of hydrogen-ion concentration, phosphorus, and calcium. The concentration of hydrogen ions was determined by the indicator method, using as guides the papers of Sorensen (7), Henderson (8), Hawk (9), and Clark (10). The buffer solutions used as standards were made by dissolving the proper amounts of mono- and di-basic sodium phosphate for alkalinity, and acetic acid and sodium acetate for acidity (8). Neutral red was found satisfactory as an indicator for the more alkaline solutions studied and methyl red for the more acid solutions. Phosphorus was determined by precipitation with molybdate reagent followed by magnesia mixture. The pyro-phosphate thus obtained was dissolved in hydrochloric acid, the solution was filtered, and the phosphoric acid was finally determined by precipitation with an excess of magnesia mixture and by ignition in the usual manner (11). Calcium was determined by precipitation with ammonium oxalate and titration of the precipitate with potassium permanganate (12).

When the plants were removed from the culture solutions, the tops were severed from the roots, cut into small pieces, dried in watch crystals at 104° C., and weighed. The roots were measured, washed free from adhering solids, and treated in the same way as the tops.

RESULTS

At the end of one week, the barley receiving the higher concentration of ferric hydrate showed a decidedly darker color and a greater growth than any of the other cultures. At this time the foliage of the peas receiving carbon black appeared somewhat darker, but the barley similarly treated was yellow, as compared with the respective control cultures.

After twenty-one days of growth the peas were just beginning to show the influence of the nutrient solutions, despite the previous removal of a large part of the reserve material of the seed. At this time the plants receiving carbon black were beginning to turn yellow and those with ferric hydrate had assumed a very dark green color.

In the barley cultures, at the end of the experiment, there was a striking contrast in the appearance of tops between the plants treated with ferric hydrate and those that had received carbon black. In the former cultures, the leaves were noticeably broader and the stems thicker than those of the control cultures, and the plants were of a very dark green color. The carbon-treated culture had produced slender plants of a yellowish green color whose general appearance was much below that of the control plants. There was a very close similarity in appearance between the plants which had received silicic acid and those of the control cultures. Those plants to which ferric hydrate had been applied appeared to have more freely branching root systems than the others, but on the whole there was a considerable uniformity in the roots of all cultures. An extra control culture of barley in distilled water plus carbon black was healthy at the end of twenty-one days, whereas the barley in distilled water alone had died some time before; hence, this added solid could hardly have been very toxic to this plant.

As more important criteria of growth than the preceding observations, the dry weights of roots and tops were measured. These results, as well as those of the study of the changes in the solution, are presented in table 1.

TABLE I

Data of Plant Yields and Analyses of Solutions for Cultures of Twenty-one Days' Duration in Knop's Solution Variously Treated

Kind of Plant	Solid Added		Yield of Dry Tops, per Plant		Yield of Dry Roots, per Plant		Length of Fresh Roots		Hydrogen-ion Concentration				Phosphorus in Solution, per Liter		Calcium in Solution, per Liter	
									Uncropped after 4 Days		Cropped, after Last 4 Days Growth		Uncropped, after 4 Days		Uncropped, after 4 Days	
	Kind	Amt.	Separate Culture	Average	Separate Culture	Average	Per Culture	Average	Per Solution	Average	Per Culture	Average	Per Solution	Average	Per Solution	Aver age
		grams	milligrams	milligrams	milligrams	milligrams	millimeters	millimeters	P_H*	P_H	P_H	P_H	milligrams	milligrams	milligrams	milli gram
Barley	None	....	97		36		215		6.0		—		86		798	
			117	107	50	43	215	215	6.0	6.0	7.4	7.4	88	87	815	806
	$Fe(OH)_3$	0.5	137		34		240									
			149	142	37	36	260	250								
	$Fe(OH)_3$	2.5	175		43		200		7.4		—		4.0		616	
			146	162	39	41	220	210	7.4	7.4	7.5	7.5	5.0	4.5	614	615
	H_2SiO_3	0.5	73		40		165									
			75	74	44	42	191	178								
	H_2SiO_3	2.5	116		59		178		6.0		—		94		812	
			88	102	50	55	162	170	6.0	6.0	7.4	7.4	84	89	790	801
	Carbon black	0.5	86		40		186									
			59	72	29	34	154	170								
	Carbon black	2.5	61		31		159		5.7		—		82		819	
			51	56	25	28	166	162	5.7	5.7	6.9	6.9	90	86	825	822
Peas	None	....	86		31		167									
			96	91	33	32	153	160								
	$Fe(OH)_3$	0.5	99		29		166									
			109	104	29	29	166	166								
	$Fe(OH)_3$	2.5	141		38		166									
			88	115	29	33	154	160								
	H_2SiO_3	0.5	71		29		114									
			56	64	22	26	108	111								
	H_2SiO_3	2.5	96		38		135									
			116	106	48	43	139	137								
	Carbon black	0.5	88		33		158									
			64	76	36	34	146	152								
	Carbon black	2.5	104		32		140									
			115	109	40	36	140	140								

* The P_H value is the number representing the logarithm of the hydrogen-ion concentration The actual hydrogen-ion concentration is higher as the P_H value decreases. Neutrality is expresse by a P_H value of 7.0. The limiting P_H values of this table, 5.7 and 7.5, represent hydrogen-io concentrations of 2×10^{-6} normal and 0.32×10^{-7} normal respectively; or 2 grams and 0.03 grams of hydrogen ions per million liters respectively.

Discussion of Results

The yield in dry weight of tops is in close agreement with the general appearance of the crops. It will be noted that the yield of tops of the barley cultures with a high application of ferric hydrate exceeded the control cultures by over fifty percent. The depression with the low concentration of silicic acid, as compared with the ineffectiveness of the high concentration, with both pea and barley plants, is interesting; however, these results are based upon the observation of too few plants to warrant any conclusion on this point.

The very decided depression in the yield of tops of barley when carbon black was applied would at first suggest the presence of toxins, in spite of the repeated washings of this material. In view of the fact, however, that the barley seedlings in distilled water, to which carbon black had been added, grew for three weeks with a good development of root system, while seedlings in distilled water alone were stunted in growth after a few days, it would seem that the carbon black was not inherently toxic. The young pea seedlings with the carbon black had an appearance of increased growth as long as they were supplied with nourishment from the cotyledons, but they began to look yellow as soon as this reserve was exhausted. This late development of the unfavorable effect would seem, therefore, to indicate some complex interaction between the plant and the solution, rather than a direct toxic effect of the carbon black.

The comparative uniformity, in all cultures, of both dry weight and length of root is in contrast with the results of Breazeale (3), who found a distinct stimulus to root growth on the addition of similar solids to cultures in toxic soil extracts. It is probable that the increase in dry weight of roots from the high application of silicic acid was due largely to small particles of the gelatinous material that could not be washed off. It will be noted that the high concentration of carbon black was accompanied by a depression of both length and dry weight of barley roots. Apparently, the increased growth of tops with ferric hydrate added to the solution was not caused by an increased surface of roots available for absorption but was due to some internal change in the plant, such as a change in the usual course of metabolism of the plant cells.

There is a consistent relation of the dry weight of tops in the various cultures to the hydrogen-ion concentration of the solution, these two values varying in opposite directions; but the variations in acidity are so small that they can hardly, in themselves, explain the differences in yield. Even the comparatively high hydrogen-ion concentration of the solution which contained carbon black was decidedly below that found by Hoagland (13) to be favorable to barley seedlings in his nutrient solutions with controlled hydrogen-ion content. He found that a hydrogen-ion concentration of 0.7×10^{-5} N. (P_H, 5.2) was not injurious, while our culture with the highest acidity was 0.2×10^{-5} N. (P_H, 5.7). Attention is called to the fact that, in all cases, the growing barley seedlings had a marked neutralizing effect on the nutrient solution, and that the solutions in which the plants had been grown were more nearly uniform in hydrogen-ion concentration than the original solutions. Hoagland (13) found that acid solutions decreased slightly in hydrogen-ion concentration, that alkaline solutions decreased markedly in hydroxyl-ion concentration, and that neutral solutions tended to remain constant during the growth of barley seedlings.

The correlation of the very small amount of soluble phosphorus in the solution containing ferric hydrate with the increased yield from that solution suggests that this paucity of soluble phosphates may be the critical factor responsible for the observed results, possibly enhancing growth by rendering the solution nearly neutral. From these results, it would seem that the nutrient solution proposed by Crone (14), using the slightly soluble ferrous and tri-calcium phosphates instead of potassium phosphate, deserves more extended trial than it appears to have received. Our results are also in accord with the observations of Truog (15) that the barley plant can get a sufficient supply of phosphorus from precipitated ferric phosphate. From the uniformity of root growth in all cultures, it would seem that we have here, not a case of the direct toxicity of the phosphate to the root, but rather a difference in the use of the materials within the plant under some difference in external conditions. Although a sufficient supply of phosphorus is necessary for the full development of plants, the presence of an excess of the phosphate ion in the culture solution would seem to be, sometimes, a limiting factor in growth. Among others, McCall (16) has also noted this effect in his sand cultures. The very dark color of the leaves of all our cultures to which ferric hydrate had been added might indicate a greater utilization of the iron under these conditions.

Lack of evidence, from the analytical data, of adsorption of nutrient elements in measurable amounts by any of the solids employed here (unless we except the small decrease of calcium in the presence of ferric hydrate) is decidedly surprising. The results of True and Oglevee (2), Breazeale (3), and Parker (4), previously mentioned, and those of McCall (16) would seem to indicate that the amounts of materials added in the present investigation should have had some detectable adsorptive effect. The last-mentioned author found that contact with even relatively coarse solids affected the physiological balance of the nutrient solution as compared with water cultures. However, the ratio of solid to solution, and the composition of both, as employed by McCall, differed from those obtaining in our work. No explanation is advanced here for the depression of yield of barley by carbon black in the apparent absence of appreciable adsorption or toxicity.

Although the results here given are based on too few data to permit of anything like conclusive deductions, some of the relations herein suggested appear to be worthy of further experimental consideration. It is suggested that such changes in the substratum as those here observed influence not merely the absorbing power of the roots but probably also the metabolism of the whole plant.

SUMMARY

1. Barley and pea plants were grown twenty-one days in a Knop's nutrient solution to which had been added: (*a*) $Fe(OH)_3$; (*b*) H_2SiO_3; (*c*) carbon black; each upon two planes of application.

2. The weight of the dry barley tops was increased approximately 50 percent by the addition of $Fe(OH)_3$ to the solution, was appreciably depressed by the addition of carbon black, and was unaffected by H_2SiO_3.

3. The weight and length of barley roots were not seriously affected by any of these added substances.

4. The weights of dry tops of barley were inversely proportional to the hydrogen-ion concentration of the solution, but the total range in the acidity of the cultures was comparatively small.

5. The growing barley plants exerted, in all cases, a neutralizing effect upon the reaction of the solution.

6. Over ninety percent of the phosphorus of the Knop's solution

was taken out of solution by the higher application of $Fe(OH)_3$, presumably by the formation of insoluble ferric phosphate. There was no evidence of poor phosphorus nutrition in this case; on the contrary, this was by far the best culture of barley, both in yield and in appearance.

7. Treatment with $Fe(OH)_3$ also produced neutrality of the nutrient medium, which may have been a factor contributing to the higher yield.

8. Peas, although deprived of a large portion of their cotyledons, had not yet developed significant differences in the various culture solutions during the growth period of twenty-one days.

9. With the proportions and kinds of solids and solutions obtaining here, there is no clear evidence of adsorption of nutrient ions.

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